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Cellulosimicrobium funkei: First Report of Infection in a Nonimmunocompromised Patient and Useful Phenotypic Tests for Differentiation from *Cellulosimicrobium cellulans* and *Cellulosimicrobium terreum*▽

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***Cellulosimicrobium funkei* is a rare, opportunistic pathogen. We describe a case of bacteremia and possibly prosthetic valve endocarditis by this organism in a nonimmunocompromised patient. Useful phenotypic tests for differentiating *C. funkei* from *Cellulosimicrobium cellulans* and *Cellulosimicrobium terreum* include motility, raffinose fermentation, glycogen, D-xylose, and methyl-α-D-glucopyranoside assimilation, and growth at 35°C.**

CASE REPORT

Clinical aspects. An 81-year-old male presented to the Casualty Department with a 2-day history of back pain, acute confusion, and fever. He had undergone a Medtronic Mosaic tissue aortic valve replacement for aortic stenosis 7 months before. On examination, he had a temperature of 39°C and looked very unwell. He had no obvious focus of infection, and systemic examination was normal. The patient had two prior admissions to the hospital in the 7 months following his operation. The first was a month after his surgery, when he was admitted to the general intensive care unit with severe pneumonia and pseudomembranous colitis. All five sets of blood cultures taken during this admission were negative, and a transthoracic echocardiogram was reported as normal. Two months after this discharge, he was readmitted and treated for presumed *Micrococcus luteus* prosthetic valve endocarditis (PVE) with 6 weeks of intravenous flucloxacillin and 2 weeks of gentamicin. Though a transesophageal echocardiogram at the time did not show any vegetation, 2 of 3 blood culture sets grew *Micrococcus luteus*. During that admission, he also developed a peripherally inserted central catheter (PICC) line infection with *Bacillus cereus*, which was treated by line removal.

One set of blood cultures was taken before patient therapy of intravenous amoxicillin-clavulanic acid at 1.2 g three times a day (TDS) commenced. After 24 h of incubation, the aerobic bottle in the blood culture set grew Gram-positive rods. Since the patient was improving clinically and a subsequent repeat blood culture was negative, it was presumed that the organism was a skin contaminant. No further identification was carried out, and the isolate was discarded. A transthoracic echocardiogram found no vegetations on the heart valves. No evidence of infection on a chest radiograph, abdominal ultrasound examination, computed tomography (CT) of the brain, magnetic resonance imaging (MRI) of the spine, or a bone scan was found. The patient received 10 days of antibiotics, during which time significant clinical improvement was noted, although his white cell count and C-reactive protein (CRP) level remained persistently elevated.

Eighteen days later, the patient developed a fever with an increasing white cell count and CRP level. Three sets of blood cultures were taken. The next day, all six bottles were positive for a Gram-positive rod. Empirical treatment for presumed prosthetic valve endocarditis was started, with intravenous vancomycin at 1 g daily and gentamicin at 80 mg twice a day (BID). An urgent transesophageal echocardiogram confirmed the presence of vegetations on the aortic valve. Antimicrobial susceptibility testing confirmed the organism's susceptibility to the patient's current antibiotics, which were continued. The patient was referred to a cardiothoracic surgeon for valve replacement. Unfortunately, the patient became increasingly drowsy over the next few days, and a CT scan of the brain demonstrated a small bleed within the right parietal lobe and an atypical ring-enhancing lesion in the left frontal lobe, suggestive of a cerebral abscess. MRI with contrast confirmed the presence of multiple lesions in the right parietal and left frontal lobes, suggestive of cerebral abscesses. In view of the intracerebral hemorrhage, surgery at this time was deemed too high risk, and conservative management with antibiotics was considered appropriate though not ideal.

Three weeks later, the patient's condition suddenly wors-

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ened and he died. A postmortem examination revealed the presence of aortic valve vegetations and cystic, hemorrhagic areas in the brain. The cause of death was stated to be prosthetic valve endocarditis. However, a specimen of the valve for microbiological confirmation could not be obtained due to medicolegal reasons (United Kingdom Human Tissue Act 2004).

Microbiology. Cell morphology obtained from the blood culture bottles showed Gram-positive rods with occasional beaded and branching forms. After 24 h of incubation at 37°C on 5% horse blood agar, colonies were 1 mm in size, nonhemolytic, yellow, convex, and smooth. The colonies penetrated into the agar upon further incubation. Cell morphology obtained from a blood agar plate showed long and slender Gram-positive rods which became coccobacillary upon further incubation. All six isolates were catalase positive and gave an API Coryne (bioMérieux) profile number 7572727, which was an "excellent identification" for *Cellulosimicrobium cellulans* (99.9%). All six isolates grew at 42°C and in the presence of 6% NaCl. However, the isolates were motile in a hanging-drop preparation, and *C. cellulans* is described as nonmotile (9). Since all six isolates were phenotypically identical, we presumed they were the same organism and sent only one isolate to the reference laboratory for confirmation of the identity.

MICs were determined by broth microdilution using a Sensititre tray (Trek Diagnostic Systems Limited, United Kingdom) incubated aerobically at 35°C (±1°C) for 24 h, as per the manufacturer's instructions. Using British Society for Antimicrobial Chemotherapy (BSAC) interpretive guidelines for corynebacteria, the isolate was recorded as resistant to penicillin (MIC of 4.0 μg/ml), erythromycin (MIC of 2.0 μg/ml), ciprofloxacin (MIC of >2.0 μg/ml), tetracycline (MIC of 4.0 μg/ml), ampicillin (MIC of 8.0 μg/ml), rifampin (MIC of >2.0 μg/ml), trimethoprim-sulfamethoxazole (MIC of 2 to 38 μg/ml), and imipenem (MIC of >16 μg/ml) but susceptible to vancomycin (MIC of <0.5 μg/ml) and gentamicin (MIC of 4.0 μg/ml).

At the reference laboratory, initial identification was made by sequencing of the 16S rRNA gene (carried out by the Molecular Identification Service, Health Protection Agency, United Kingdom). A partial sequence of 635 bp of the patient's isolate was obtained (GenBank accession number HQ402902), corresponding to positions 344 to 1030 of the coding sequence. Related sequences were found by a BLAST search of GenBank, with the results showing that the sequence matched the type strains of *C. cellulans* at 99.99% (only 1 base different), *Cellulosimicrobium funkei* at 100%, and *Cellulosimicrobium terreum* at 98.2%. Therefore, we could not distinguish between *C. cellulans* and *C. funkei* by this method, as previously described by Brown et al. (1), but *C. terreum* (11) could be excluded. Yoon et al. showed that *C. terreum* shares only ~97% sequence identity with the 2 other species, but our partial sequence of 635 bp showed an increased percentage of similarity (11). As there are currently no other phylogenetically relevant genes described in the literature, such as *rpoB* for this genus, and DNA-DNA hybridization is not available to us, the species had to be distinguished by phenotypic tests. The only phenotypic tests readily available were motility and raffinose fermentation testing (1). As the isolate was motile and did not ferment raffinose, it was presumptively identified as *C. funkei* (1). We obtained the type strains of *C. cellulans* (DSM 43879), *C. funkei* (DSM 16025), and *C. terreum* (DSM 18665) from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany, for comparison.

TABLE 1. Phenotypic characteristics that differentiate the clinical isolate and *C. funkei* type strain from the *C. cellulans* and *C. terreum* type strains

Characteristic	Strain			
	Clinical	*C. funkei*	*C. cellulans*	*C. terreum*
Growth at 42°C	+[a]	+	+	−
Growth at 35°C	+	+	+	−
Motility	+	+	−	−
Acid from:				
Raffinose	−	−	(+)	−
Ribose	+	+	+	−
Glycogen	+	+	+	−
API CH50 assimilation				
Glycogen	+	+	−	−
D-Xylose	−	−	+	−
Arbutin	+	+	+	−
MDG[b]	+	+	−	+

[a] +, positive; −, negative; (+), weak positive.
[b] Methyl-α-D-glucopyranoside.

The type strains were also compared with the patient's isolate by whole-cell fatty acid (CFA) analysis, API CH50 assimilation tests, and the API Coryne (bioMérieux) identification system. In API CH50 assimilation tests using AUX medium, strains were incubated at 30°C for 72 h, and results were read again after a further 5 days of incubation. There were no changes in test results after the initial 72 h of incubation. For CFA analysis, cultures were grown on Trypticase soy broth agar (TSBA; Becton Dickinson Co., United Kingdom) at 30°C aerobically for 2 days. The CFAs were extracted and analyzed by gas chromatography (MIDI Sherlock, Newark, NJ) using the MIDI system's rapid TSBA database.

Using the API Coryne (bioMérieux) system, all 4 strains were inoculated as per manufacturer's instructions, with an additional strip inoculated with *C. terreum* incubated at 30°C. No changes in test results with *C. terreum* incubated at 37°C and at 30°C were found. Although *C. terreum* does not grow at 35°C or above, the concentration of cells in the suspension (turbidity of a McFarland standard of 6 or above) meant that positive results were obtained at 37°C after 24 h due to enzyme activity without the necessity for the organism to grow.

Table 1 compares the differential biochemical tests of the patient's isolate and the type strains.

Table 2 shows the CFA profiles of all 4 isolates. There were not enough differences for this method to be useful to differentiate between the 3 species.

Since *C. funkei* is described as being urease positive and our isolate was negative in the API Coryne kit, we retested urease production using Christensen's urea slope. The type strain of *C. funkei* and the clinical isolate gave weak or negative results when tested on three different occasions. The urease test was repeated, with incubation at 24°C, 30°C, and 36°C for 5 days. The clinical isolate gave a very weak positive result at the

TABLE 2. CFA composition of our clinical isolate and type strains *C. funkei*, *C. cellulans*, and *C. terreum*

Fatty acid[a]	CFA composition (%)[b]			
	Clinical isolate	Type strain of:		
		C. funkei	*C. cellulans*	*C. terreum*
Anteiso-$C_{13:0}$	0.1		0.5	0.2
Iso-$C_{14:0}$	1.1	1.0	4.5	0.53
$C_{14:0}$	7.0	6.3	6.1	3.6
Anteiso-$C_{15:1}$ A	0.7		0.5	0.1
Iso-$C_{15:0}$	8.0	10.4	4.9	10.7
Anteiso-$C_{15:0}$	52.6	52.8	46.0	47.29
Iso-$C_{16:0}$	6.2	5.2	18.7	13.1
$C_{16:0}$	15.7	12.5	11.30	6.8
Iso-$C_{17:0}$	0.5	0.8	0.2	0.8
Anteiso-$C_{17:0}$	6.9	8.0	4.7	10.14
$C_{17:0}$	0.1	0.2	0.5	0.3
$C_{18:0}$	0.2	0.3	0.3	1.0
Similarity index to *C. cellulans*	0.808	0.902	0.833	0.353

[a] Fatty acids that represent <0.5% for all 4 strains have been omitted.
[b] CFA composition analyses cannot be used to discern these species.

lowest temperature only, the type stain of *C. funkei* a very weak positive result at 30°C, and the rest remained negative.

Our isolate was deposited in the National Collection of Type Cultures as NCTC 13535.

Cellulosimicrobium species are Gram-positive rods of the family *Promicromonosporaceae*, suborder *Micrococcineae*, and are widely distributed in soil and water (4, 10). The genus *Cellulosimicrobium* was first proposed by Schumann et al. in 2001 for the bacterium previously classified as *Cellulomonas cellulans* (basonym *Nocardia cellulans*, embracing the misclassified species *Cellulomonas cartae*, *Brevibacterium lyticum*, *Brevibacterium fermentans*, and *Oerskovia xanthineolytica*) (9). The rationale for this proposal was the distinct position of this species on the neighbor-joining phylogenetic tree, based on 16S rRNA gene sequencing, and the presence of a unique peptidoglycan in the cell wall, which was absent from authentic *Cellulomonas* species. This genus comprised only the type species *C. cellulans* until 2006, when Brown et al. proposed that some clinical isolates previously identified as *Oerskovia turbata* be included in this genus as a new species, C*ellulosimicrobium funkei*, based on 16S rRNA sequencing and chemotaxonomic analysis (1). Subsequently, in 2007, Yoon et al. proposed a third species, *Cellulosimicrobium terreum*, as identification for an isolate from soil from Dokdo, South Korea, that had 16S rRNA sequencing similarity of 97.4 to 97.6% to both *C. cellulans* and *C. funkei* (11) Because *C. terreum* is not that closely related genetically and it does not grow at and above 35°C, it is unlikely to be misidentified as either of the other 2 species by gene sequencing or phenotypic testing. However, the description of *C. terreum* is based on just one strain, and it is possible that other strains could grow closer to 35°C than the type strain and therefore could potentially be found in clinical samples. In such cases, a failure to grow at 42°C, lack of motility, and inability to ferment ribose and glycogen (both present in the API Coryne kit [bioMérieux]) could help distinguish *C. terreum* from the other 2 species.

Brown et al. (1) found that the main phenotypic differences between the type strains of *C. cellulans* and *C. funkei* were that the latter is motile, does not ferment inulin or raffinose, and is sensitive to imipenem but resistant to trimethoprim-sulfamethoxazole (1). Our isolate was resistant to imipenem, suggesting this may not be a useful identifying characteristic. We also found that assimilation testing for glycogen, methyl-α-D-glucopyranoside, and D-xylose were useful. Our isolate and that of *C. funkei* assimilated glycogen and methyl-α-D-glucopyranoside but not D-xylose, in contrast to *C. cellulans*, which had opposite reactions. Unusually, our isolate was urease negative and reduced nitrate to nitrite. Brown et al., in their description of *C. funkei* sp. nov., showed the type strain to be urease positive and negative for nitrate reduction. However, the three additional *C. funkei* strains they tested reduced nitrate. This was reflected in their emended description of the genus, which states that nitrate reduction can be positive or negative (1). Results for urease production may be negative or slowly and weakly positive, depending on whether the API Coryne system or Christensen's urea slant, respectively, is used. Obviously, more strains of the 3 species than were available to us need to be tested to confirm the reliability of these tests for species differentiation.

The API Coryne (bioMérieux) identification system does not have *C. funkei* or *C. terreum* in its database. Until the database is changed to include the newer species and reflect additional testing necessary for correct identification, isolates are likely to be misidentified as *C. cellulans* and infections by *C. funkei* may be underrecognized. Hence, all isolates should be sent to a reference laboratory for confirmation of identity.

Cellulosimicrobium funkei is an uncommon, opportunistic pathogen. Our search found only two reported cases of infection with this organism, and both cases were found in immunocompromised patients. The first was endocarditis, originally reported as an *Oerskovia turbata* endocarditis, found in a 68-year-old male on high-dose prednisolone for Crohn's disease and ankylosing spondylitis who had a prosthetic aortic valve. The infection was associated with a contaminated homograft solution (1, 6, 7) The second was a central venous catheter (CVC)-related bacteremia found in a patient with acute myelogenous leukemia and reported as *Oerskovia turbata* bacteremia (1, 5). Unfortunately, we could not confirm the presence of this organism on the aortic valve in our patient. However, we believe that since this organism was isolated from all 6 bottles of 3 blood culture sets in the presence of proven endocarditis, it is most likely to be the causative organism. Our patient endured 2 infections of his mosaic tissue valve over several months, involving *Micrococcus luteus* and *Cellulosimicrobium funkei.* The Medtronic Mosaic tissue valve is a supra-annular stented porcine tissue valve in which the leaflets have been fixed at low pressure in a predilated aortic root and covered with an amino-oleic acid demineralization treatment to improve its hemodynamic performance and durability. Though we could not find any studies that looked at the propensity for biofilm formation on this type of heart valve compared to that of other prosthetic valves, clinical studies comparing it to the Carpentier-Edwards porcine valve, Freestyle valve, homograft, and Ross procedure have not found a higher rate of endocarditis with the Mosaic valve (2, 3).

There is an excellent review of infections caused by *Cellulosimicrobium* and *Oerskovia* species in the literature (8).

To conclude, *Cellulosimicrobium funkei* is a rare, opportunistic pathogen in immunocompromised patients. This was a first report of infection in a nonimmunocompromised host. The API Coryne (bioMérieux) commercial identification system does not reflect recent changes in taxonomy and is likely to misidentify isolates as *C. cellulans*. 16S RNA gene sequencing identifies the genus and can differentiate *C. terreum* from *C. funkei* and *C. cellulans* but cannot distinguish between the latter two species. CFA composition analyses cannot be used to discern these species either. Hence, additional phenotypic testing should be carried out. Susceptibility to imipenem may not be a reliable phenotypic test for differentiating *C. funkei* from *C. cellulans*. However, motility, D-xylose, glycogen, and methyl-α-D-glucopyranoside assimilation, and raffinose fermentation testing may be useful and need to be used on additional strains.

Nucleotide sequence accession number. The partial sequence of the patient isolate described above is available in GenBank under accession number HQ402902.

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